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Cytokines and biological markers in autoimmune GFAP astrocytopathy: The potential role for pathogenesis and therapeutic implications

Akio Kimura[a,*], Masao Takemura[b], Yasuko Yamamoto[c], Yuichi Hayashi[a], Kuniaki Saito[c], Takayoshi Shimohata[a]

[a] Department of Neurology, Gifu University Graduate School of Medicine, Gifu, Japan
[b] Advanced Diagnostic System Research Laboratory, Fujita Health University, Aichi, Japan
[c] Department of Disease Control and Prevention, Fujita Health University, Aichi, Japan

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ABSTRACT

Autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy (GFAP-A) is a corticosteroid-responsive meningoencephalomyelitis with a poorly understood pathogenesis. We examined and compared the levels of cytokines and biological markers in the cerebrospinal fluid (CSF) of patients with GFAP-A and other neurological disorders. We identified four cytokines (tumor necrosis factor alpha [TNFα], Interleukin [IL]-27, IL-6, and chemokine [C-C motif] ligand 20) and three biological markers (GFAP, S100 calcium-binding protein B, and neurofilament light chain) present at elevated levels in CSF samples during the acute phase of GFAP-A. Additionally, we identified significant correlations between CSF TNFα, IL-27, IL-6, and CSF biological markers.

1. Introduction

Autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy (GFAP-A) was recently reported as a spectrum of autoimmune inflammatory central nervous system (CNS) disorders characterized by detection of immunoglobulin G (IgG) against GFAP in cerebrospinal fluid (CSF) (CSF GFAP-IgG) by both tissue and cell-based testing (Fang et al., 2016; Flanagan et al., 2017). This disorder manifests as a distinctive corticosteroid-responsive meningoencephalomyelitis with lymphocytic pleocytosis, which is often accompanied by a hallmark linear perivascular radial gadolinium enhancement in the brain detected by magnetic resonance imaging (Flanagan et al., 2017; Shan et al., 2018; Zekeridou et al., 2018; Kunchok et al., 2019). Since its discovery, GFAP-A has been reported in several countries, though its pathogenesis is poorly understood (Yang et al., 2017; Dubey et al., 2018; Iorio et al., 2018; Long et al., 2018; Yang et al., 2018; Zarkali et al., 2018; Kimura et al., 2019). As predicted by GFAP's intracellular location, GFAP-specific cytotoxic T cells are likely effectors of this disorder as CSF GFAP-IgG does not have pathogenic potential (Flanagan et al., 2017; Zekeridou et al., 2018). CSF GFAP-IgG is a biomarker for immune inflammation (Shan et al., 2018). Previous work has reported that GFAP-reactive $CD8^+$ T cells can avoid tolerance mechanisms and, depending on the T cell-triggering event, drive unique aspects of inflammatory CNS autoimmunity (Sasaki et al., 2014). Unlike patients with well-characterized cytotoxic T cell–mediated autoimmune disorders, patients with GFAP-A are steroid responsive. Flanagan et al. speculated that other inflammatory components of the immune system, including microglia, macrophages, cytokines, and chemokines, in addition to CD8+ T cells, contribute to GFAP-A pathogenesis (Flanagan et al., 2017). We considered that cytokines play important roles for GFAP-A pathogenesis, and therefore investigated the characteristics of CSF cytokine profiles in this disorder. Additionally, we examined CSF biological markers, including GFAP and S100 calcium-binding protein B (S100B), which are astrocytic damage markers, and neurofilament light chain (NFL), which is a neuronal damage marker. Furthermore, we examined the associations between cytokines and biological markers to elucidate their pathogenic roles in GFAP-A.

2. Patients and methods

2.1. Patients

In this study, we compared the CSF levels of cytokines and biological markers among patients with GFAP-A, other autoimmune CNS disorders, infectious CNS disorder, and psychosomatic disorders (PSD). We examined CSF samples from patients with multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) to represent other autoimmune CNS disorders, varicella zoster virus meningitis

* Corresponding author at: Department of Neurology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan.
E-mail address: kimura1@gifu-u.ac.jp (A. Kimura).

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(VM) as an infectious CNS disorder. We enrolled 79 patients with these neurological disorders and PSD that were admitted to our hospital (Department of Neurology, Gifu University Graduate School of Medicine, Gifu, Japan) between October 2002 and October 2018. Among these 79 patients, 14 had GFAP-A [median age: 44; age range: 18–66; men:women = 8:6], 25 had MS [median age: 45; age range: 26–65; men:women = 11:14], 14 had anti-aquaporin 4 (AQP4) antibody-positive NMOSD [median age: 51; age range: 29–79; men:women = 0:14], 11 had VM [median age: 63; age range: 22–82; men:women = 7:4], and 15 had PSD [median age: 27; age range: 15–70; men:women = 9:6]. The 14 GFAP-A patients were described previously (Kimura et al., 2019). CSF samples were collected from all 79 patients and stored at −30 °C until analyzed. All CSF samples from patients with MS, NMOSD, and VM were collected during the acute phase and before treatment. Likewise, CSF samples from GFAP-A patients were collected during the acute phase. However, samples from two GFAP-A patients were collected after the administration of steroid therapy; one sample was collected 4 days after treatment with dexamethasone (32 mg/day for 2 days) and the other was collected 3 days after treatment with intravenous methylprednisolone (1000 mg/day for 3 days). We obtained informed consent from all patients for the secondary use of CSF samples. This study was approved by the institutional review board of Gifu University Graduate School of Medicine, Gifu, Japan.

2.2. Detection of CSF GFAP-IgG

We investigated the CSF GFAP-IgG using both transfected cell-based and tissue-based immunofluorescence assays. We performed the cell-based assay as previously reported (Flanagan et al., 2017). Human embryonic kidney 293 cells were transfected with a plasmid from OriGene (Rockville, MD) that encodes a single *Homo sapiens* GFAP transcript variant 1 (RG204548; pCMV6-AC-GFAP-α-GFP). Transfected cells were fixed with 2% paraformaldehyde for 20 min and permeabilized with phosphate-buffered saline (PBS) containing 0.2% Triton X-100 and 1% bovine serum albumin for 10 min. The fixed cells were incubated with 1% bovine serum albumin/PBS at 4 °C overnight to block nonspecific IgG binding. Cells were then exposed to patient CSF (1:4) for 1 h, washed in PBS, then exposed to Cy3-conjugated donkey anti-human IgG (1:1500, Jackson ImmunoResearch; West Grove, PA) for 1 h. We performed the immunohistochemical analysis with ready-to-use frozen rat brain sagittal sections (RF-201-SS; Zyagen, CA) and GFAPα autoantibody-positive patient CSF samples. Frozen brain sections were fixed with 4% paraformaldehyde at room temperature for 15 min and permeabilized with 0.2% Triton X-100 in PBS for 10 min. After washing in PBS, normal goat serum (10%) was applied for 30 min to block nonspecific IgG binding. The sections were then incubated with diluted patient CSF (1:4) at 4 °C overnight. After washing in PBS, the sections were incubated with Alexa-Fluor 488 goat anti-human IgG (1:1500, Molecular Probes; Eugene, OR) for 1 h.

2.3. Analysis of CSF cytokines and biological markers

A magnetic human cytokine multiplex assay (Human Th17 Magnetic Bead Panel, Millipore, MA) was used to measure 25 cytokines: interleukin (IL)-17F, granulocyte macrophage colony-stimulating factor, interferon (IFN)γ, IL-10, chemokine (C-C motif) ligand 20 (CCL20), IL-12P70, IL-13, IL-15, IL-17A, IL-22, IL-9, IL-1β, IL-33, IL-2, IL-21, IL-4, IL-23, IL-5, IL-6, IL-17E, IL-27, IL-31, tumor necrosis factor (TNF)α, TNFβ, and IL-28A. This assay was performed according to the manufacturer's instructions using diluted patients' CSF (1:2).

Human enzyme-linked immunosorbent assay (ELISA) kits were used to measure the levels of CSF GFAP (RD192072200R, BioVendor, Czech Republic), S100B (RD192090100R, BioVendor, Czech Republic), and NFL (SEE038Hu 96 Tests, Cloud-Clone Corp, TX). These assays were performed according to the manufacturer's instructions using diluted patients' CSF (1:2).

2.4. Statistical analysis

Data analysis was performed using statistical software (Excel-Toukei 2012, Social Survey Research Information; Tokyo, Japan). Statistically significant differences in levels of cytokines and biological markers among the five patient groups were calculated using the Steel-Dwass test. Twenty-one out of twenty-five cytokines tested in GFAP-A patients had median values below the lowest limit of detection (LLOD). The other four cytokines (CCL20, IL-6, IL-27, and TNFα) were detected in GFAP-A patients and we performed statistical analyses on these cytokine levels among patient groups. However, these cytokine levels were below the LLOD in some patient groups. Supplementary table 1 summarizes the number and percentage of patients whose samples were below the LLOD for each cytokine. When this occurred, these values were replaced with half the assay LLOD for statistical analysis, as done in previous reports (Vexler et al., 2015; Gupta et al., 2017). Spearman's rank correlation was used to assess the correlations among these four cytokines, biological markers, other CSF findings (cell counts and protein levels), and GFAP-A patient outcomes. A P-value $\leq .05$ was considered statistically significant.

3. Results

3.1. Detection of CSF GFAP-IgG and clinical features of GFAP-A

We used a cell-based assay to detect autoantibodies against GFAPα in CSF samples from all 79 patients and identified these autoantibodies in 14 patients with GFAP-A, but not other patients (Fig. 1A–D). Additionally, CSF from GFAP-A patients showed immunoreactivity against astrocytes in pial, subpial, and periventricular regions of rat brains (Fig. 1E and F). Ovarian teratoma was identified in two of these patients, one of which had anti-*N*-methyl-D-aspartate receptor (NMDAR) antibodies in CSF. We summarized the demographics of these GFAP-A patients in Table 1. Details regarding the clinical features of these GFAP-A patients were described previously (Kimura et al., 2019).

3.2. Comparison of CSF cytokine levels among patient groups

Table 2 summarizes the CSF cytokine levels among the five patient groups. Of the 25 tested cytokines, we identified elevated levels of four cytokines (CCL20, IL-6, IL-27, and TNFα) in GFAP-A patient CSF samples. We compared these levels among patient groups in Fig. 2. The level of CSF TNFα in the GFAP-A patient group was significantly higher than in the other patient groups (MS: $P < .0000001$; NMOSD: $P = .0001$; VM: $P = .007$; PSD: $P < .0001$). Additionally, the level of CSF IL-27 was significantly higher in the GFAP-A patient group than in other patient groups (MS: $P < .00001$; NMOSD: $P = .03$; VM: $P = .01$; PSD: $P < .0001$). GFAP-A patients had significantly higher levels of CSF CCL20 than other patients, excepting those with VM (MS: $P = .003$; NMOSD: $P = .002$; PSD: $P = .0005$). Finally, GFAP-A patients had significantly higher levels of CSF IL-6 than MS and PSD patients (MS: $P = .001$; PSD: $P = .02$).

3.3. Comparison of CSF biological marker levels among patient groups

Table 3 summarizes the levels of CSF biological markers among the five patient groups. We compared these levels among patient groups in Fig. 3. We found that the GFAP-A patient group had significantly higher levels of CSF S100B than other patient groups (MS: $P < .00001$; NMOSD: $P = .008$; VM: $P = .0006$; PSD: P = .0001). The level of CSF GFAP was significantly higher in the GFAP-A patient group than in other patient groups, excepting the NMOSD patient group (MS: $P < .000001$; VM: $P = .0003$; PSD: P < .0001). Similarly, we found CSF NFL levels were significantly higher in GFAP-A patients than in

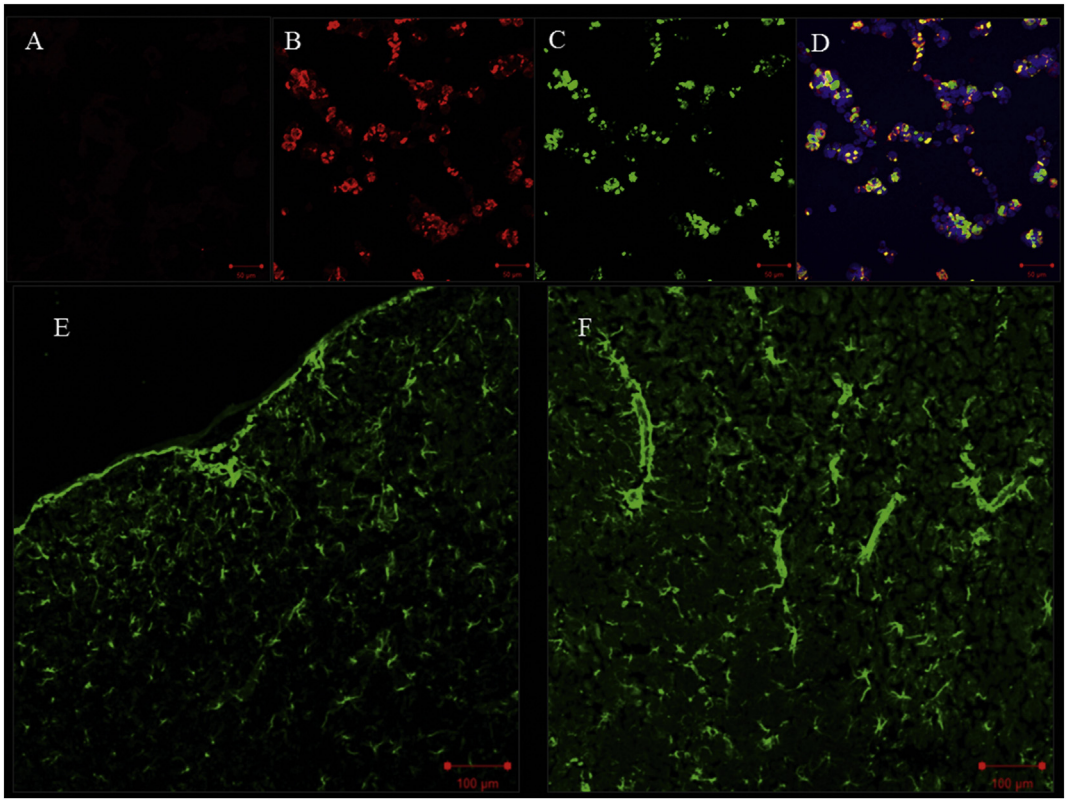

Fig. 1. Detection of CSF GFAP-IgG by both transfected cell-based and tissue-based immunofluorescence assays.
Cell-based assay of glial fibrillary acidic protein (GFAP) α-transfected human embryonic kidney (HEK) 293 cells (A–D). HEK293 cells stably expressing green fluorescent protein (GFP)-tagged GFAPα (C D). GFAP IgG was detected in the cerebrospinal fluid (CSF) of patients with autoimmune GFAP astrocytopathy (1:4, B), but not in the CSF of a control patient (1:4, A). Colocalization of patient IgG and GFAPα is yellow in merged images (D). DNA is stained with 4,6-diamidino-2-phenylindole (blue). Tissue-based immunofluorescence assay using frozen rat brain sagittal sections (E, F). Immunoreactivity of patient CSF-IgG was observed in astrocytes of the pial, subpial (E), and perivascular regions (F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Demographics of patients with autoimmune GFAP astrocytopathy.

	Median [range]	Patients, Number (%)
Number		14
Age	44 [18–66]	
Female		6 (43)
Complicated tumor		
Ovarian teratoma		2 (14)
Coexisting autoantibody		
Anti-NMDAR antibody in CSF		1 (7)
Cerebrospinal fluid findings		
Cell counts (/μL)	142 [15–364]	
Protein levels (mg/dL)	192 [71–320]	

CSF: cerebrospinal fluid, GFAP: glial fibrillary acidic protein, NMDAR: *N*-methyl-D-aspartate receptor.

other patients, excepting those with NMOSD (MS: P < .00001; VM: P = .003; PSD: $P = .0002$).

3.4. Correlations between cytokines and biological markers, and other CSF findings

Table 4 presents correlations between cytokines (CCL20, IL-6, IL-27, and TNFα) and biological markers (GFAP, S100B, and NFL), and other CSF findings (cell counts and protein levels). Between cytokines and biological markers, we found that the CSF TNFα level correlated with the CSF GFAP level ($R = 0.635$, $P = .015$), S100B level ($R = 0.940$, $P < .1 \times 10^{-5}$), and NFL level ($R = 0.706$, $P = .005$). The CSF IL-6 level correlated with the CSF GFAP level ($R = 0.538$, $P = .048$), S100B level ($R = 0.891$, $P < .2 \times 10^{-4}$), and NFL level ($R = 0.750$, $P = .002$). The CSF IL-27 level correlated with the CSF S100B level ($R = 0.614$, $P = .02$) and NFL level ($R = 0.541$, $P = .046$). Additionally, the level of CSF IL-27 correlated with the level of CSF protein ($R = 0.543$, $P = .045$). Between each cytokine, we identified significant correlations among levels of TNFα, IL-27, and IL-6 (TNFα vs. IL-27: $R = 0.600$, $P = .023$; TNFα vs. IL-6: $R = 0.847$, $P < .001$; IL-27 vs. IL-6: $R = 0.589$, $P = .027$).

4. Discussion

In this study, we described the cytokines and biological markers in the CSF of patients with GFAP-A, and the association between them. We obtained novel findings of GFAP-A, as follows. First, GFAP-A displays a profile of cytokines and biological markers distinct from MS, NMOSD, VM, and PSD. Second, we identified four cytokines (TNFα, IL-27, IL-6, and CCL20) and three biological markers (GFAP, S100B, and NFL) present at elevated levels in CSF samples during the acute phase of GFAP-A. Finally, we identified significant correlations between CSF TNFα, IL-27, IL-6, and CSF biomarkers, and identified significant correlations among levels of these three cytokines.

TNFα and IL-6 are proinflammatory cytokines and, based on their correlation with GFAP, S100B, and NFL, may be associated with astrocytic and neuronal cell damage (Allan and Rothwell, 2001; Olmos and Lladó, 2014). The cell types expressing these cytokines are unknown. However, several reports described the histopathological findings of GFAP-A (Iorio et al., 2018; Long et al., 2018; Shu et al., 2018). Iorio et al. reported on histopathological analysis of a leptomeningeal biopsy that revealed a necrotizing inflammatory process with infiltrate,

Table 2
CSF cytokine profiles of patients with GFAP-A, MS, NMOSD, VM, and PSD.

Cytokines	GFAP-A	MS	NMOSD	VM	PSD
IL-17F (ng/mL)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
GM-CSF (ng/mL)	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06
IFN-γ (pg/mL)	< 10	< 10	< 10	< 10	< 10
IL-10 (pg/mL)	< 1	< 1	< 1	< 1	< 1
CCL20 (pg/mL)	31.4 (27.6–36.9)	18.8 (13.7–26.2)	13.1 (2.5–17.7)	29.2 (17.3–37.9)	10.7 (5.39–14.7)
IL-12P70 (pg/mL)	< 5	< 5	< 5	< 5	< 5
IL-13 (pg/mL)	< 7	< 7	< 7	< 7	< 7
IL-15 (pg/mL)	< 5	< 5	< 5	< 5	< 5
IL-17A (pg/mL)	< 12	< 12	< 12	< 12	< 12
IL-22 (ng/mL)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
IL-9 (pg/mL)	< 9	< 9	< 9	< 9	< 9
IL-1B (pg/mL)	< 5	< 5	< 5	< 5	< 5
IL-33 (pg/mL)	< 5	< 5	< 5	< 5	< 5
IL-2 (pg/mL)	< 12	< 12	< 12	< 12	< 12
IL-21 (pg/mL)	< 5	< 5	< 5	< 5	< 5
IL-4 (ng/mL)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
IL-23 (ng/mL)	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
IL-5 (pg/mL)	< 6	< 6	< 6	< 6	< 6
IL-6 (pg/mL)	9.58 (1.25–31.2)	< 2.5	15.4 (2.10–185)	< 2.5	< 2.5
IL-17E (ng/mL)	< 0.12	< 0.12	< 0.12	< 0.12	< 0.12
IL-27 (ng/mL)	1.69 (1.37–2.93)	0.17 (0.11–0.30)	0.65 (0.54–0.94)	0.46 (0.24–1.18)	0.12 (0.08–0.21)
IL-31 (ng/mL)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
TNF-α (pg/mL)	22.9 (12.2–57.2)	< 2.5	< 2.5	< 2.5	< 2.5
TNF-β (ng/mL)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
IL-28A (ng/mL)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04

Median (interquartile range) data values are shown.
GFAP-A: autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy, CCL20: chemokine (C-C motif) ligand 20, GM-CSF: granulocyte macrophage colony-stimulating factor, IFN: interferon, IL: interleukin, MS: multiple sclerosis, NMOSD: neuromyelitis optica spectrum disorder, PSD: psychosomatic disorders, TNF: tumor necrosis factor, VM: varicella zoster virus meningitis.

characterized by the presence of CD8+ lymphocytes, macrophages, and some multinucleated giant cells (Iorio et al., 2018). Long et al. reported that brain biopsies from four cases of GFAP-A showed inflammatory infiltrates (lymphocytes, monocytes, and neutrophils) surrounding blood vessels and microglial activation (Long et al., 2018). It is possible that activated macrophages or lymphocytes produce these cytokines (Cuturi et al., 1987; Ratner and Clark, 1993). However, a different report found that activated astrocytes release proinflammatory cytokines (Aschner, 1998; Brambilla, 2019). It is possible that dysfunctional astrocytes, rendered so by CD8+ T cells, release these proinflammatory cytokines.

Previous studies report that activated astrocytes in patients with MS and experimental autoimmune encephalomyelitis (EAE) secrete chemokines, including CCL20, that signal continued recruitment of immune cells into the CNS (Ambrosini et al., 2003; Ambrosini et al., 2005; Arima et al., 2012). It was also reported that both active and passive immunization against CCL20 improve EAE and are potential therapies for MS (Abraham et al., 2017). Our recent case-series study on GFAP-A demonstrated that many patients experience lymphocytic pleocytosis lasting for several months (Kimura et al., 2019). In GFAP-A, activated astrocytes could secrete CCL20 and further recruit lymphocytes into the CNS, leading to the lymphocytic pleocytosis observed in patients. However, astrocytes can secrete anti-inflammatory cytokines, including IL-27, that behave as immunosuppressants and play a role in suppressing the proinflammatory activation of leukocytes and microglia, thereby favoring tissue repair and neuroprotection (Sénécal et al., 2016; Brambilla, 2019). Although GFAP-A is likely effected by CD8+ T cells, GFAP-A patients have a relatively good prognosis. Therefore, we propose that the elevated level of CNS IL-27 behaves as an immunosuppressant and leads to tissue repair and neuroprotection. However, further examination of these cytokines, including cellular locations and longitudinal changes, are needed to clarify the pathogenic role these cytokines play in GFAP-A.

We observed elevated levels of astrocytic damage markers in the GFAP-A patient group. This finding is consistent with a previous report that the CSF GFAP level in GFAP-A patients was significantly higher than in patients with other autoimmune and inflammatory neurological disorders in the CNS (Yang et al., 2019). In this study, we identified that the CSF level of S100B in GFAP-A patients was not only significantly higher than in patients with MS, VM, and PSD, but was also significantly higher than in patients with NMOSD as well. This result may indicate that the degree of astrocytic damage during the acute phase was more severe in GFAP-A than in NMOSD, which is an autoimmune astrocytopathy mediated by anti-AQP4 autoantibody and complements. Interestingly, we observed an elevated level of NFL, a neuronal injury marker, in the GFAP-A patient group. A previous report of neuropathological GFAP-A findings described neuron loss in addition to astrocyte loss (Long et al., 2018). However, another report described no astrocyte pathological alteration (no loss of GFAP) and no signs of demyelination (no loss of myelin oligodendrocyte glycoprotein) (Shu et al., 2018). Though each GFAP-A case may present a different degree of injury, it is possible that neurons, in addition to astrocytes, are injured by immune attack. Considering these results, we suggest that early administration of immunotherapy is important to avoid irreversible damage and sequelae.

A limitation of this study is that many cytokines we examined were present at levels below the LLOD. Although our study identified increased CSF levels of four cytokines (CCL20, IL-6, IL-27, and TNFα), it is possible that the CSF levels of other cytokines, including those we could not examine in this study, may increase during the acute phase of GFAP-A. It will be necessary to measure the levels of more cytokines using more sensitive assays to better understand GFAP-A pathogenesis.

Approximately 20–50% of GFAP-A patients have relapsing courses (Flanagan et al., 2017; Yang et al., 2017; Long et al., 2018; Kunchok et al., 2019). To prevent relapses, some GFAP-A patients required the administration of oral prednisone over a long period of time. However, our recent study demonstrated that half the GFAP-A patients had a good prognosis, despite receiving no oral prednisone after steroid pulse therapy (Kimura A, et al. 2019). It is important to establish biomarkers to evaluate appropriate corticosteroid therapy for GFAP-A. CSF GFAP-IgG is essential for diagnosis but is not a suitable biomarker for evaluation of corticosteroid therapy. The cytokines and biological markers

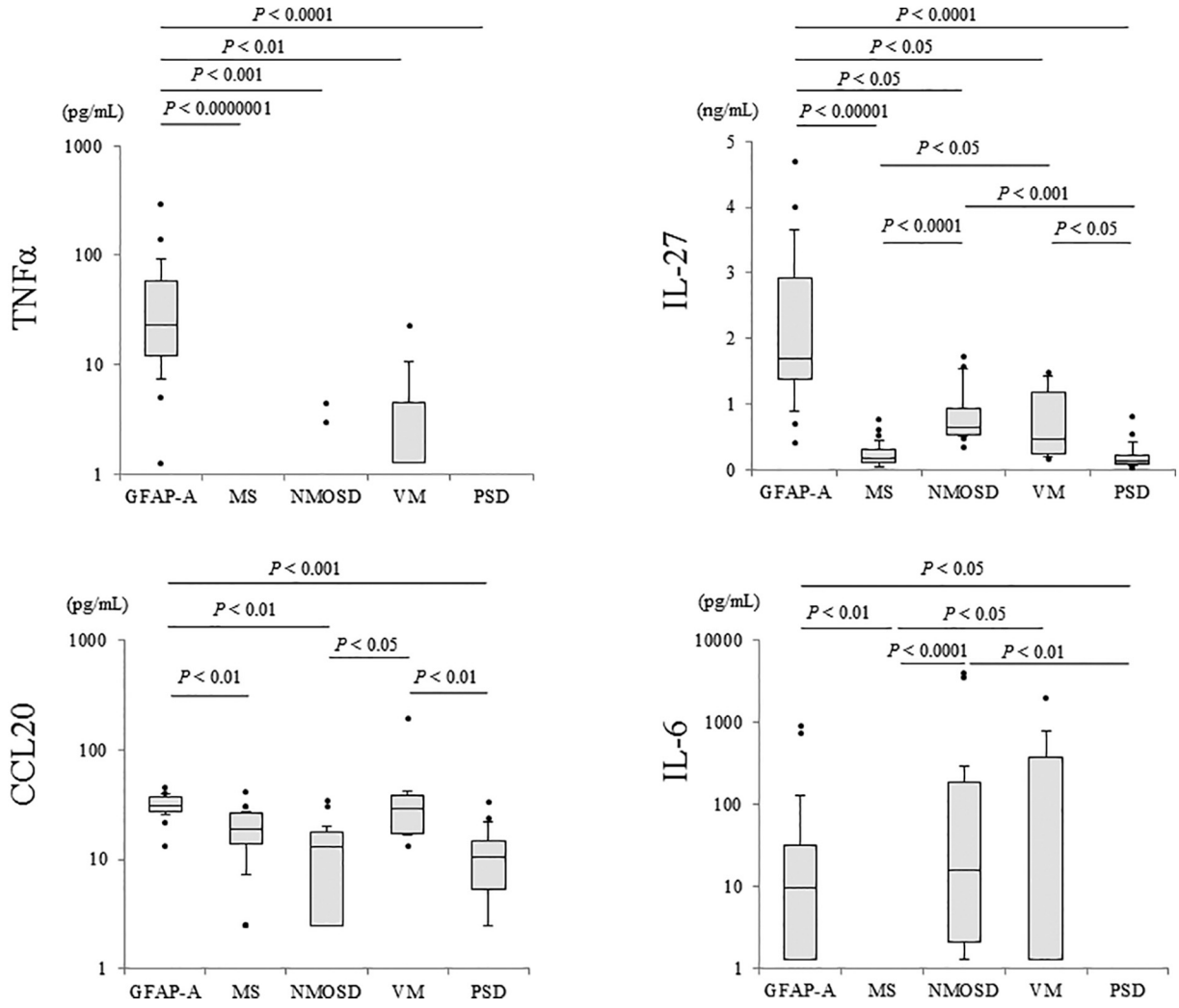

Fig. 2. Comparison of CSF cytokine levels among patient groups.
The plots show the 90th percentile (bars), 75th and 25th percentiles (box), and median (bar in box). GFAP-A, autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy; CCL, chemokine (C-C motif) ligand; CSF, cerebrospinal fluid; IL, interleukin; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; PSD, psychosomatic disorders; TNF, tumor necrosis factor; VM, varicella zoster virus meningitis. Black dots: outliers.

Table 3
CSF biological marker profiles of patients with GFAP-A, MS, NMOSD, VM, and PSD.

Markers	GFAP-A	MS	NMOSD	VM	PSD
GFAP (ng/mL)	48.7 (28.0–62.7)	0 (0–0.03)	28.8 (3.23–32.9)	1.02 (0.23–2.08)	0 (0–0)
S100B (pg/mL)	922 (405–1510)	0 (0–85)	164 (106–284)	125 (95.1–190)	0 (0–19.5)
NFL (pg/mL)	300 (244–877)	69.9 (52.7–107)	121 (98–300)	110 (91.5–177)	36 (22.0–54.2)

Median (interquartile range) data values are shown.
GFAP-A: autoimmune glial fibrillary acidic protein astrocytopathy, GFAP: glial fibrillary acidic protein, MS: multiple sclerosis, NFL: neurofilament light chain, NMOSD: neuromyelitis optica spectrum disorder, PSD: psychosomatic disorders, S100B: S100 calcium-binding protein B, VM: varicella zoster virus meningitis.

characterized in this study may be useful biomarkers for GFAP-A. Further studies are needed to clarify the relationships between these cytokines, biological markers, and the clinical outcomes of GFAP-A patients.

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Competing interests

The authors have no competing interests to declare.

Author contribution

AK designed the study, assayed the biological markers, analyzed the data, and drafted the manuscript. YH collected the data. MT, YY, and KS assayed the cytokines. TS supervised the study. All authors read and approved the final manuscript.

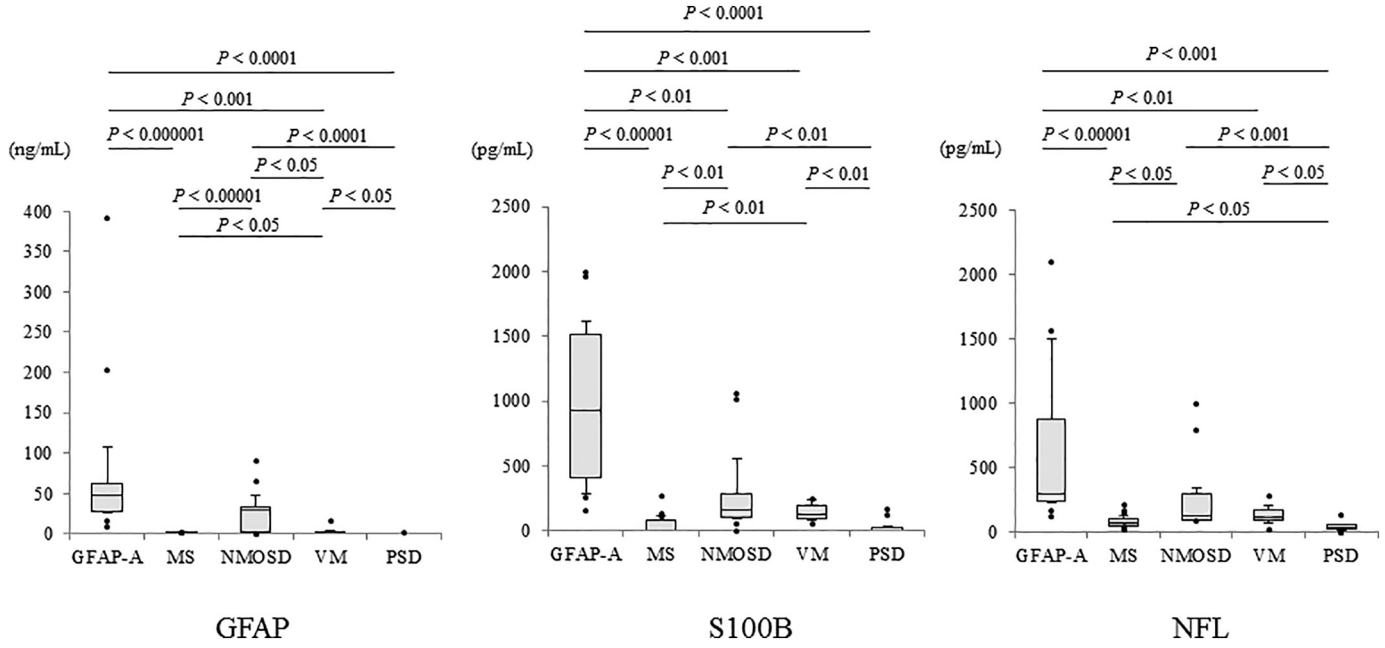

Fig. 3. Comparison of CSF biological marker levels among patient groups.
The plots show the 90th percentile (bars), 75th and 25th percentiles (box), and median (bar in box). GFAP-A, autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy; CSF, cerebrospinal fluid; MS, multiple sclerosis; NFL, neurofilament light chain; NMOSD, neuromyelitis optica spectrum disorder; PSD, psychosomatic disorders; S100B, S100 calcium-binding protein B; VM, varicella zoster virus meningitis. Black dots: outliers.

Table 4
Correlations between cytokines and biological markers, and other CSF findings in autoimmune GFAP astrocytopathy.

	Cerebrospinal fluid findings								
	Cell counts	Protein	GFAP	S100B	NFL	TNF-α	IL-27	CCL20	IL-6
TNF-α	$R = -0.354$ $P = .215$	$R = 0.358$ $P = .209$	**R = 0.635** **P = .015**	**R = 0.940** $\boldsymbol{P < .1 \times 10^{-5}}$	**R = 0.706** **P = .005**	–	**R = 0.600** **P = .023**	$R = 0.362$ $P = .203$	**R = 0.847** **P < .001**
IL-27	$R = 0.191$ $P = .513$	**R = 0.543** **P = .045**	$R = 0.292$ $P = .311$	**R = 0.614** **P = .020**	**R = 0.541** **P = .046**	–	–	$R = 0.209$ $P = .474$	**R = 0.589** **P = .027**
CCL20	$R = 0.213$ $P = .464$	$R = 0.090$ $P = .759$	$R = 0.376$ $P = .185$	$R = 0.500$ $P = .069$	$R = 0.029$ $P = .923$	–	–	–	$R = 0.176$ $P = .547$
IL-6	$R = -0.462$ $P = .096$	$R = 0.251$ $P = .386$	**R = 0.538** **P = .048**	**R = 0.891** $\boldsymbol{P = .2 \times 10^{-4}}$	**R = 0.750** **P = .002**	–	–	–	–

CCL20: chemokine (C-C motif) ligand 20, GFAP: glial fibrillary acidic protein, IL: interleukin, S100B: S100 calcium-binding protein B, TNF: tumor necrosis factor, NFL: neurofilament light chain.
Bold values indicate significant correlations ($P < 0.05$).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2019.576999.

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